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METABOLIC RATES IN FIVE ANIMAL POPULATIONS IN 1977 AFTER
PROTONGED EXPOSURE TO SEAFARER ELF ELECTROMAGNETIC
FIELDS IN NATURE.

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Bernard Greenberg and Noreen Ash Department of Biological Sciences University of Illinois at Chicago Circle Chicago, Illinois 60680

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Report for Summer 1977

Prepared for

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OFFICE OF NAVAL RESEARCH BRANCH OFFICE CHICAGO
536 South Clark Street
Chicago, Illinois 60605

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FOREWORD

This study was performed under Contract No. NO0014-77-C-0331 for the Office of Naval Research.

The authors thank J. R. Gauger and W. F. Lancaster of the field staff of IIT Research Institute (IITRI) for electric and magnetic field measurements.

Respectfully submitted,

Dr. B. Greenberg, Professor

Department of Biological Sciences

University of Illinois at Chicago

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1. SUMMARY

During the summer of 1977, five species of animals were collected under the U.S. Navy's Extremely Low Frequency (ELF) antenna at the Wisconsin Test Facility (WTF) while controls were collected seven to thirteen miles away from the antenna. Exposed and control animals of the same species were tested for oxygen consumption and respiratory quotient (R.Q.) on the day of collection at the WTF. The species include one vertebrate, the redbacked salamander, Plethodon cinereus cinereus (Green), and four invertebrates: the earthworm, Lumbricus terrestris L.; the redworm, Lumbricus rubellus (Hoffmeister); the woodlouse, Oniscus asellus L.; and the slug, Arion sp., There were no statistically significant differences in the oxygen consumption or R. Q. between exposed and control groups in any species except for a significant depression in the oxygen consumption of exposed redworms (P <.001). Oxygen consumptions were statistically analyzed over the years (1972, 1973, 1975, 1976, and 1977) and showed no significant differences between exposed and control groups in any of the five species. Our field observations and sampling of the five exposed populations continue to show no abnormalities in behavior, habitat selection, or external features and pigmentation after eight years of WTF operation

2. INTRODUCTION

Since the program was initiated in the summer of 1972, there have been annual collections of four invertebrates and a vertebrate for the purpose of analyzing their oxygen consumptions and respiratory quotients (R.Q.). Although representing four diverse phyla, all share the common attribute of leading a relatively sedentary life on or in the soil thereby being closely coupled with the ELF electrical and magnetic fields. The animals are the redbacked salamander, Plethodon cinereus cinereus (Green), the woodlouse, Oniscus asellus L., the slug, Arion sp., the earthworm, Lumbricus terrestris L., and the redworm, Lumbricus rubellus (Hoffmeister).

In practice, metabolism is generally measured in terms of the animal's oxygen consumption, although carbon dioxide or heat production would serve equally well. One may refer to the oxygen consumption of the animal as a whole in terms of oxygen consumption/unit time (total metabolism) or the animal's weight may be taken into account as we have done in this study, i.e. oxygen consumption/unit weight/unit time (metabolic rate). The metabolic rates of poikilotherms depend on a number of extrinsic and intrinsic factors such as temperature (Gelineo, 1964), humidity (Templeton, 1960), level of activity (Mill, 1972), previous temperature adaptation (Kanungo and Prosser, 1959a), season and/or time of day (Stern and Mueller, 1972), reproductive cycle (Prosser and Brown, 1962), quantitative changes in enzymatic systems (Kanungo and Prosser, 1959b), body weight (Dawson and Bartholomew, 1956), etc.

Although oxygen consumption varies with the situation, it is a highly reliable measurement provided a rigorous experimental design is followed.

Consistent with this rigorous design, test and control specimens have been collected on or close to the same date each year, tested about the same time of day, and always tested simultaneously. This year additional variables were eliminated by collecting exposed and control animals on the same day they were tested at WTF, avoiding variables associated with shipping and holding.

Over fifty comparisons of oxygen consumptions and respiratory quotients of exposed and control populations have been made from 1972 to 1976. R.Q.'s have never differed significantly while in three instances statistical tests have revealed differences in oxygen consumption:

(1) in 1972 there was a marginal elevation in oxygen consumption of exposed woodlice (P <.05); (2) in fall of 1974 oxygen consumption was lower in exposed redworms (.05> P >.025); and (3) in 1976 it was higher in exposed redworms (.005 >P >.001). After seven years of WTF antenna operation, with the antenna operating about twenty per cent of the time, our studies revealed no sustained alterations in metabolism of animals subjected to prolonged exposure (Greenberg, 1974; Greenberg and Ash, 1976a, b; Greenberg, 1977). The results of the 1977 monitoring program with the antenna in operation seventy-nine per cent of the time, are presented here.

3. MATERIALS AND METHODS

3.1 Collection of Animals

Exposed animals were collected at the same site used in previous years (Greenberg and Ash, 1976a, b), along the north leg of the antenna, just south of Highway 77. Controls were collected on the same day seven to thirteen miles from the nearest WTF antenna at the same sites as in previous years (Fig. 1). Collection dates for each species are: woodlice, June 24; earthworms, July 12; salamanders, July 26; slugs, August 12; and redworms, August 19. All specimens were collected before 11:00 A.M. and immediately taken to the environmental laboratory at WTF and tested. Exposed and control animals of the same species were tested simultaneously.

3.2 Oxygen Consumption and Respiratory Quotient

Oxygen consumption was measured in a closed manometric system with a Gilson differential respirometer set at 20°C. Animals were washed, dried, and weighed to the nearest 0.01 gm on a Mettler scale and then placed in 15-ml Warburg flasks with one sidearm. Salamanders were tested one per flask while others were grouped as follows to maximize reliability of the system: woodlice, ten; redworms, three; earthworms, three; and slugs, three. Specimens were grouped for uniformity of weight. Woodlice and salamanders were not sexed; worms and slugs are hermaphroditic. One-tenth ml of a .5M KOH solution was introduced into the sidearm as a CO2 absorbent, and the center well was partially filled with distilled water to raise the humidity within the flask and it was screened to exclude the animals. The flasks were allowed to equilibrate for 15

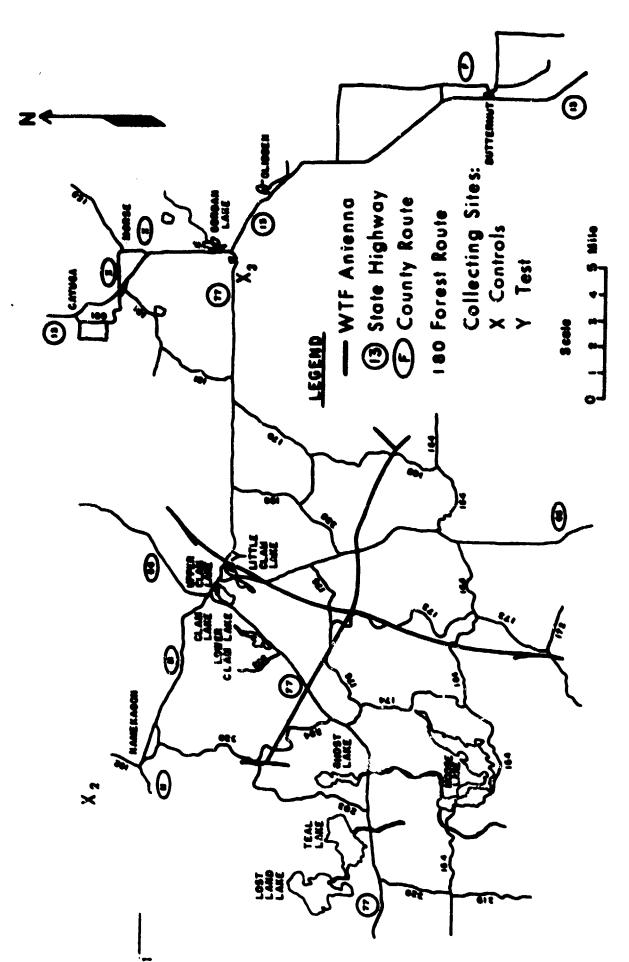


Fig. 1. Collection Sites of Exceed and Control Animals.

minutes and readings were taken every 15 minutes. When volumes of 0_2 consumed were roughl, equal for two consecutive periods, readings were made for an additional two or three periods, giving a total of four or five consecutive readings as a basis for mean 0_2 consumption. Metabolic rate is expressed as $\mu 1$ 0_2 consumed/gm/hr. Volume of CO_2 evolved during respiration was determined by titration of .2N HCl against the alkaline absorbent plus 1 ml saturated BaCl2 and 1 ml .1% methyl orange. To obtain R.Q., $\mu 1$ CO_2 was calculated using the gas laws.

3.3 Electric and Magnetic Field Measurements

The meters employed to measure the output voltages of the probes described here were the specially constructed tuned voltmeters (TVM's) which were supplied by the Navy for ELF measurements, the commercially available Hewlett-Packard 302A wave analyzer, and the commercially available Hewlett-Packard 358lA signal wave analyzer. All three instruments function as battery operated, frequency selective voltmeters. The TVM's and HP302A were used from 1972 through 1975, and the HP358lA was used in 1976 and 1977. The HP 358lA is a newly available instrument, and was factory modified for a 1 Hz bandwidth and battery operation. It was used to replace the increasingly unreliable TVM's and the bulky HP302A. The HP358lA was used in its 30-Hz bandwidth mode to measure the MSK signal. A multi-frequency battery operated notch filter, which was designed and fabricated by IITRI, was used in conjunction with the HP358lA to eliminate power line interference at 60 Hz and the harmonics thereof when taking MSK measurements.

The magnetic field was measured using a single axis magnetic field probe designed and built by IITRI. This probe is merely a many-turned coil with a ferrite core and terminating resistor. Appropriate conversion

factors were used to convert the voltage reading at the output of the probe to an equivalent magnetic flux density.

The low impedance electric field (i.e., the horizontal electric field at the earth's surface) was measured with one-meter probe wires.

The sensor used for measuring the high impedance electric field (i.e., the field in air) was the IITRI constructed ELF electric field probe. The probe consists of two insulated hemispheres which form a split sphere 6 cm in diameter. The probe senses the electric field on its axis and transmits a modulated light replica of the electric field to the receiver via a six-foot fiber optic cable. At the far end of the cable, the specially designed receiver changes the light signal back to an electric signal which is proportional to the field sensed by the transmitter. The output of the receiver is measured with a frequency selective voltmeter and converted by means of appropriate conversion factors to an electric field intensity.

Each of the three field probes is sensitive on a single axis.

Therefore, accurate and repeatable methodologies for measuring the field magnitudes were needed. Two cases were apparent: (1) the mode of a single frequency being transmitted by a single antenna and (2) the mode of both antennas transmitting the same MSK signal at some relative phase.

When the WTF transmits single frequency/single antenna, the electric and magnetic fields generated at any point may be thought of as a simple vector having unique magnitude and direction. It should be noted that in this case the field magnitude in a direction perpendicular to the direction of the field vector is zero. Therefore, the method used to determine the magnitude of the fields in 1972 to 1976 was as

follows:

- a) Three orthogonal components of each field were measured; i.e., the north-south, east-west, and vertical components (only the north-south and east-west components of the low impedance electric field were measured as this field has no vertical component at the earth's surface)
- b) The magnitude of each field was then computed as the root of the sum of the squares of its orthogonal components.

When the two antenna elements are operated simultaneously, the electric and magnetic fields generated are more complex. The two sources contribute components to the total fields which, in general, are out of phase. The fields cannot be described by a single measurement unless one of the sources dominates, as is the case for many of the measurements made. Therefore, the method used in 1977 was to search for the maximum and minimum fields at each location.

3.4 Wisconsin Test Facility Operations

In the years preceding 1977, all measurements were made with the WTF transmitting single frequency, sinusoid signals at 45 Hz and 75 Hz. Measurements were made for each frequency and for each leg of the antenna transmitting separately. These conditions reflected the primary operating modes of the WTF up to that time. August 1976, however, the WTF commenced twenty-four hour operation for almost one-third of the time with both antennas transmitting a minimum shift keying (MSK) signal with a center frequency of 76 Hz and a modulation rate of 16 Hz. MSK is a special type of frequency modulation (FM). The electromagnetic field measurements in 1977, therefore, were made with the WTF operating in this condition. The schedule for August 1976 through August 1977 is summarized in Table 1. The N/S and E/W antennae operated a total of 7488 and 7526 hours, respectively.

TABLE 1. WTF OPERATION, AUGUST 1976 THROUGH AUGUST 1977.

	NOPTH	SOUTH A	ANTENNA	EAST/W	EST A	NTENNA	DAYS OF CONTINUOUS OPERATION
Month	HRS.	MIN.	PERCENT	HRS.	MIN	PERCENT	
AUG.1976	442	23	59.5	494	27	66.5	8/5,8/13 8/17,8/18, 8/21,8/22,8/24,8/25
SEPT. ²	709	38	98.5	685	01	95.1	9/4 to 9/27
ост.	668	45	89.9	643	38	86.5	10/2 to 10/9,10/14, 10/15,10/28 to 10/31
nov.	557	40	77.5	557	4C	77.5	11/1 to 11/3, 11/7
DEC.	530	51	71.4	530	51	71.4	NONE
JAN.1977	558	52	75.1	563	55	75.8	1/24 to 1/31
FEB.	557	24	82,9	571	14	85.0	2/1 to 2/3,2/25 to 2/28
MARCH	654	41	88.0	654	40	83.0	3/1,3/3,3/4,3/10 to 3/21
APR IL	581	45	80.8	583	45	81.1	4/18 to 4/24
MAY	630	58	84.8	638	22	85.8	5/2 to 5/8,5/26,5/27
JUNE	422	03	58.6	538	46	74.8	NONE
JULY	580	34	78.0	474	04	63.7	7/7,7/8,7/10,7/15, 7/16
AUG.	592	15	79.5	590	02	79.3	8/14 to 8/22
TOTAL	7487	49	78.84	7526	25	79.3	110

^{1.} East/West Antenna only.

^{2.} South underground cable section included in operations on Sept. 4 and thereafter.

^{3.} North/South Antenna only.

Mean of monthly percentages.

3.5 Statistical Treatment

The 0_2 consumption and R.Q. data were analyzed using a single classification analysis of variance for unequal sample sizes. The 0_2 consumption data were tested across the years utilizing a t-test for paired comparisons to test whether the mean of the annual differences between exposed and control populations varies significantly from a hypothetical mean of zero. The 95% confidence limits for the mean were computed using \underline{t} and the standard deviation of the mean.

4. RESULTS

4.1 Electric and Magnetic Field Data

Table 2 gives the measured magnetic flux densities at the animal collecting sites during 1972-1977. The values show a certain degree of fluctuation from year to year. The reason for this is that the only locations where the field exceeds the 0.001 gauss level are physically near an antenna. At these locations, positioning of the probe is very important. Near the antenna, the magnetic fields may be expected to vary as the inverse of the distance from the test point to the antenna. As a result of this behavior, the highest values of magnetic field occur closest to the antenna; but at these locations, the positioning is also most critical. This fact is borne out by measurement. The higher magnetic flux densities show a larger degree of change than the lower magnetic flux densities. This is due to the fact that it was difficult to obtain exactly the same measurement locations and positioning of the probe year after year.

The maximum 1977 field values, although measured with both antennas transmitting a MSK signal are quite similar to maximum field levels obtained in previous years with single antenna, single frequency measurements. This is not an unexpected result, as most of the siter are situated near only one antenna, and hence this antenna will contribute most of the field component at the site. The 1977 minimum field values were generally less than 0.001 gauss.

Both the low impedance electric field (the horizontal electric field at earth's surface) and the high impedance electric field (the field in air) have been measured at each of the test and control plots.

TABLE 2

MAGNETIC FIELDS AT TEST AND CONTROL PLOTA,

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The low impedance electric field has been measured each year since 1972. The high impedance electric field was measured in 1975 because of interest in fields of this type under high voltage lines, and to more completely define the electric field environment for the test and control plots.

The low impedance electric field (the horizontal electric field at the earth's surface) was measured with one-meter probe wires.

Table 3 gives the measured low impedance electric fields for the period 1972-1977.

The 45- and 75-Hz readings show some fluctuation that may be explained, in part, by uncertainty in placement of the sensor. However, since the electric field varies as the natural logarithm of the inverse of the distance from the antenna, this cannot account for all of the differences from year to year. The main explanation for the yearly variations in these data is that the low impedance electric field is more affected by the differences in the earth's conductivity and other factors such as nearby long conductors which occur between measurements.

The maximum electric field levels measured in 1977 are similar to the field levels measured in previous years due to the operation of the antenna element nearest the measurement site. The minimum electric field levels for 1977 were generally on the order of the field levels measured in previous years due to the operation of the antenna element farthest from the measurement site. Again, these results are not unexpected as the antenna element nearest a measurement point will dominate the field levels at that point.

TABLE 3

LOW IMPEDANCE ELECTRIC FIELDS AT THE TEST AND CONTROL PLOTS.

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The 60-Hz fields are quite variable because they depend, in large part, upon conditions which cannot be controlled. The 60-Hz field depends on the current in nearby power lines, and the amount of current allowed to flow in power system neutrals. Of all these factors, the only one that remains relatively constant from year to year is the placement of the lines. Even this may change, however, as power systems are upgraded and expanded.

4.2 Biological Data

Table 4 summarizes mean oxygen consumption (µ 1/gm/hr) with 95% confidence limits of exposed and control animals tested in summer 1977. The redworms are the only species of the five tested to exhibit a statistically significant difference (P < .001) between exposed and control animals, viz. a marked lowering in the exposed animals. There were no significant differences in R.Q. of test and control groups (Table 6).

Table 5 and Figures 2 through 6 give a six-year summary of oxygen consumption. The 1974 populations are not included in the t-test for paired comparisons depicted in Table 5 as they were collected in fall rather than summer. Some perspective on the question of a seasonal change in the sensitivity of animals to ELF electromagnetic fields may be noted in the graphs and has been discussed previously (Greenberg, 1976a). When examined on a long-range basic (Table 5) none of the species show significant differences between the exposed and control populations. However, when the absolute differences between the exposed and control redworm populations are examined, it appears that these populations are diverging. Since these fluctuations are not consistent it is difficult to postulate a biological mechanism

which would explain these differences in population response.

As can be seen from the graphs in 1977, three of the exposed groups and all five of the control groups showed higher $\mathbf{0}_2$ consumption rates than in previous years. We attribute some of the previous lower values to shipping effects.

There was no evidence of abnormalities in behavior, habitat selection or external features and pigmentation in any of the animals collected in the proximity of the WTF antenna.

TABLE 4

HEAN OXYUEN (NOTIANA SHOC	HEAN OXYGEN CONSUMPTION (, 1/g) WITH 957, CONFIDENCE LIDITS OF EXPOSED AND CONTROL AND MILS, SUPPER 1977.	ITS OF EXPOSED AND	CONTROL ANDVALS, SUPPORT 1977
ORCANISM	, OM	EXPOSED ANIDALS! MEAN \$2 CONSUMPTION	жо.	CONTROL ANTHALS' MEAN 02 COMBURTION
WOODLICE	8	324.59 (305.31 - 343.87)	100	305.29 (283.86 - 326.72)
EARTH-HORMS b	27	128.46 (116.25 - 140.67)	27	138.94 (119.20 - 158.68)
SALAMANDERS	10	104.91 (85.88 - 123.94)	10	115.39 (100.86 - 129.92)
STUCS	21	247.31 (237.39 - 257.23)	27	240.12 (213.61 - 266.63)
REDHORMS	77	115.92 (85.11 - 146.73)	54	191.27 (163.68 - 218.86)

a There were no significant differences between exposed and control animals except for redworms (P <.001).

b Weight range = 0.4 to 0.83 g per specimen.

c Weight mange = 0.6 to 1.4 g per specimen.

TABLE 5

MEAN OXYGEN and 1977 ^a .	CT-TEST FOR	(u 1/g) 10P EXC PAIRED COMPAR	POSED AND ISONS).	CONTROL ANTHA	S IN THE	Summers of	MEAN OXYCEN CONSUMPTION (u 1/g) TOF EXPOSED AND CONTROL ANIMALS IN THE SUMMERS OF 1972, 1973, 1975, 1976, and 1977 ^a . (T-TEST FOR PAIRED COMPARISONS).
ORGANISM		1972	1973	1975	1976	1977	EXPOSED VS CONTROL
HOODLICE	Test Control	235.00 205.86	229.80 222.00	172.88 170.44	245.33 263.73	324.59 305.29	n. sig. (.4> P> .2)
earthforms ^b	Test Control	119.67 123.33	85.90 81.49	123.22 126.44	113.78 124.68	128.46 138.94	n. sig. (.2> P> .1)
SALAMANDERS ^C	Test Control	97.99	76.25 85.05	89.87 76.39	108.53 94.04	104.91	n. sig. (P> .75)
STACS	Test Control	134.83 120.67	117.40	128.04 138.88	197.73 204.28	247.31 240.12	3. s1g. (P~.5)
REDWORMS	Test Control	171.62 173.59	138.22 135.94	145.78 163.98	166.81 127.88	115.92 191.27	n. sig. (gg)

In 1974 θ_2 Consumption was monitored in fall not summer.

Weight range = 0.4 to 0.8 g per specimen.

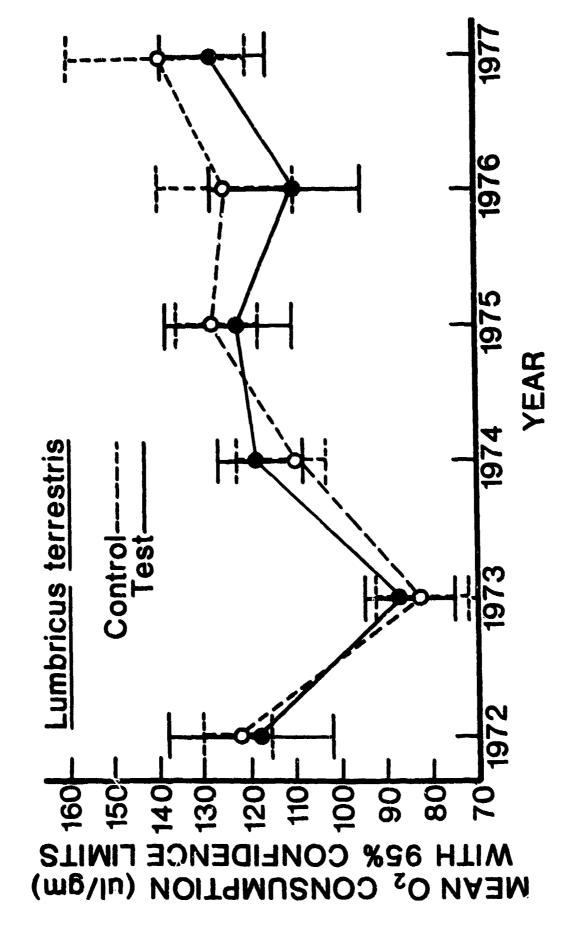
Weight Fange = 0.6 to 1.4 g per specimen.

TABLE 6

MEAN RESPIRATORY QUOTIENTS WITH 95% CONFIDENCE LIMITS OF EXPOSED AND CONTROL ANIMALS⁴, SUPPLE 1977.

ORGANISM	EXPOSED	CONTROL
MOODLICE	0.81 (0.74-0.88)	0.80 (0.72-0.88)
EARTHMORMS	0.80 (0.73-0.87)	0.82 (0.76-0.88)
SALAMANDERS	0.82 (0.75-0.89)	0.81 (0.75-0.87)
stucs	0.80 (0.74-0.86)	0.78 (0.71-0.85)
REDWORMS	0.82 (0.76-0.88)	0.81 (0.76-0.86)

There were no significant differences between exposed and control animals of the same species.



Mean Oxygen Consumption of the Earthworm, Lumbricus terrestris, 1972-77, F18. 2.

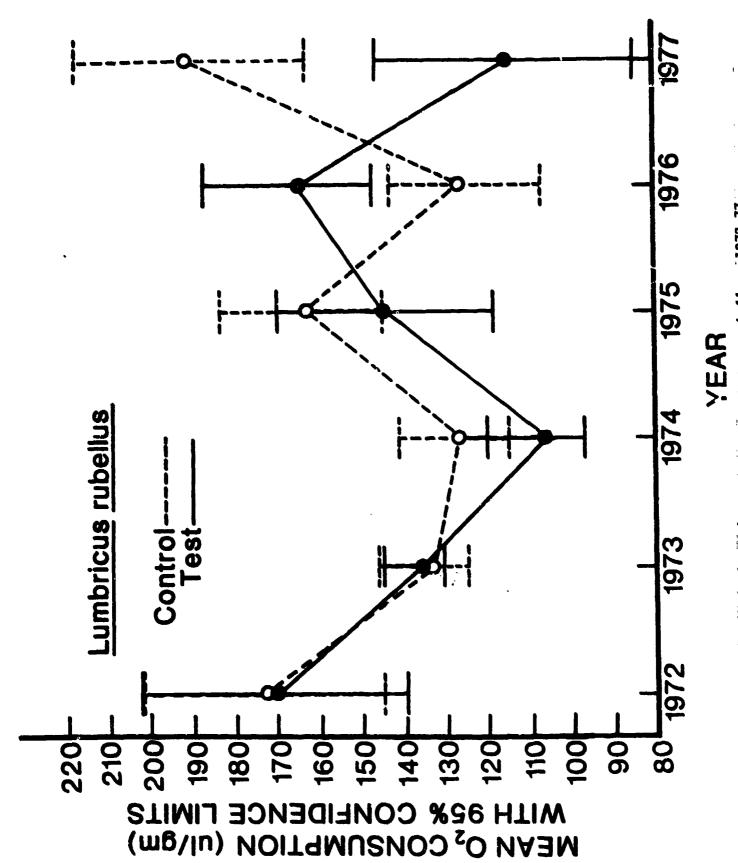


Fig. 3. Mean Oxygen Consumption of the Redworm, Lumbricus rubellus, 1972-77

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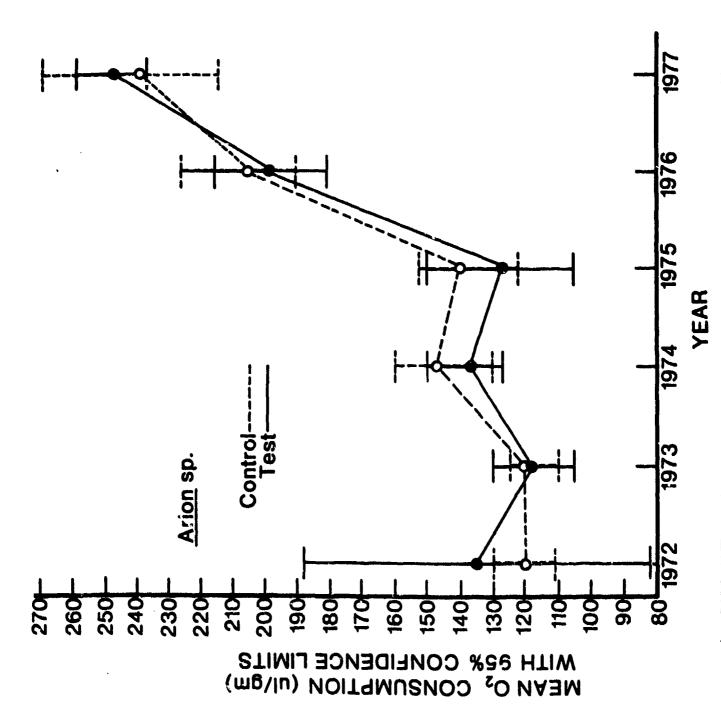


Fig. 4. Mean Oxygen Consumption of the Slug, Arion sp., 1972-77.

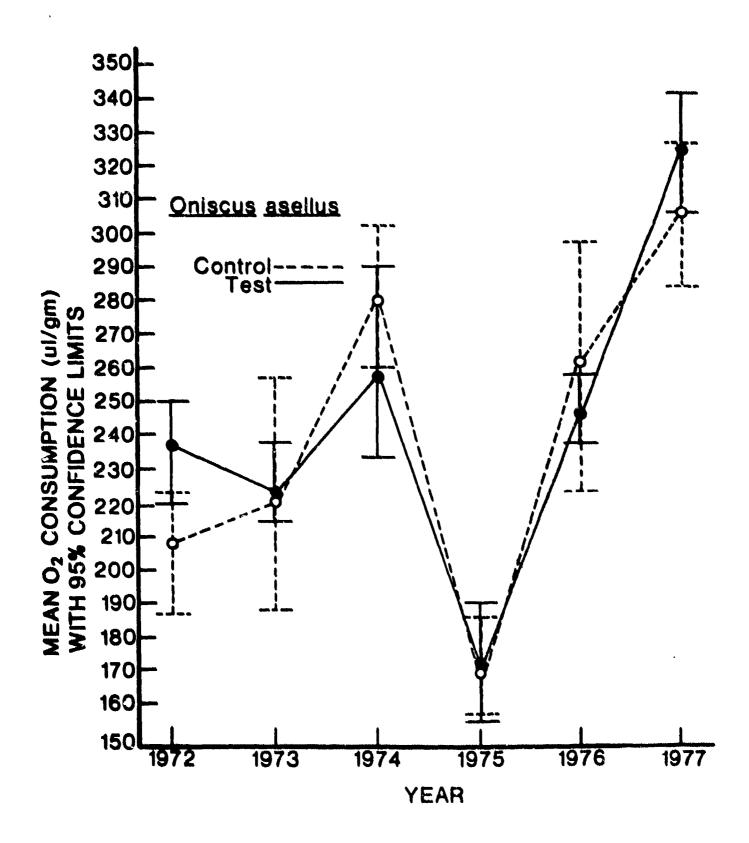


Fig. 5. Mean Oxygen Consumption of the Woodlouse, Oniscus asellus, 1972-77.

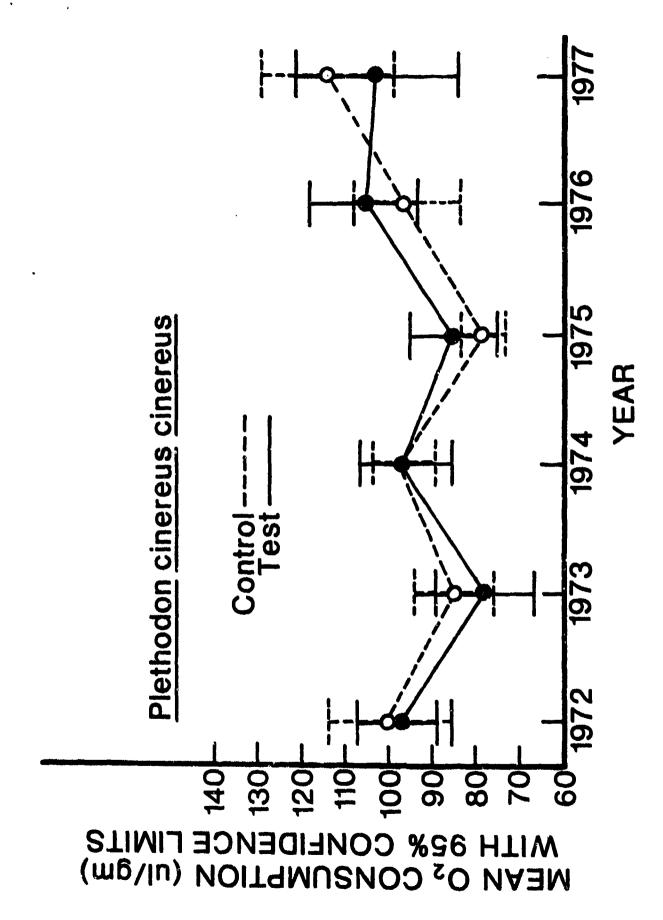


Fig. 6. Mean Oxygen Consumption of the Redbacked Salamander, Plethodon cinereus cinereus, 1972-77.

5. DISCUSSION

From the study's inception in summer, 1972, up to and including summer, 1976, we shipped specimens to our Chicago laboratories for testing; shipment was via air or car, but always identical for control and exposed animals of the same species. The specimens were shipped in ice chests and conserved at 12°C after arrival, the total time elapsing between collection and testing rarely exceeding 48 hours. We found that different modes of shipping produced demonstrable differences in oxygen consumption but control and exposed animals did not differ (Greenberg and Ash, 1976). Nevertheless, it is possible to argue that these animals did have different metabolic rates (presumably due to the Suafarer electromagnetic environment) and that packing, shipping, and cooling wiped out these differences. Poikilotherms may complete their acclimation to cool temperatures in one day (Mellanby, 1939) and their oxygen consumption depends upon previous temperature adaptation (Montuori, 1907). Therefore, in summer 1977 a Gilson differential respirometer and other apparatus were installed at the Wisconsin test facility for same-day, on-site measurements of oxygen consumption. It is not surprising, therefore, to note in Figures 2 to 6 that all five of the control groups and three of the exposed groups had higher oxygen consumptions in 1977 than in previous years. This release from a probable depressent effect of shipping and cooling is most pronounced in woodlice and slugs and least pronounced in salamanders.

Although the on-site method of testing probably yields data closer to the true values than the former method, both methods clearly show that the oxygen consumptions of paired populations of

exposed and control slugs, salamanders, woodlice, and earthworms still continue to show no statistically significant differences after eight years of WTF antenna operation and a total of 25 tests. The last year was of particular importance because the facility was operating at 79 per cent time compared with about 20 per cent in previous years. During the same year, temperature and rainfall were near normal for this area (Table 7 and 8). Significant differences in oxygen consumption appeared for the third time in redworm populations. This year the metabolic rate in exposed worms was depressed, as it was in fall 1974, whereas in 1976 the reverse was true. Significant differences in redworm metabolic rates have been detected three out of six years and the differences between the exposed and control groups are becoming progressively greater over the years as evidenced by the significant positive regression. Nevertheless, neither the exposed nor the control populations have been consistently elevated or depressed. The fluctuations are such that when the data are tested over the years via a t-test, there is no significant difference between exposed and control redworms. In 1977, oxygen consumption of exposed redworms was the lowest since we started testing in 1972, and highest in the controls. We have no explanation for the ditterences and the inconsistencies in the oxygen consumption of control and exposed redworms.

We conclude from the evidence presented that the Seafarer electromagnetic environment has not had a demonstrable effect on the respiratory quotients of five species of animals studied since

1972. Nor has it had a demonstrable effect on the metabolic rates of woodlice, slugs, earthworms, or redbacked salamanders. Data from our long-term study of redworm metabolic rates do not exclude a possible ELF effect.

TABLE 7

MEAN MAXIMIM AND MINIMIM TEMPERATURES (^OC) FOR APPROXIMATELY 30 DAYS BEFORE COLLECTION OF SAMPLES.

		1972	1973	73	13	1975	1976	9/	1977	7.1
SAMPLE	ž	Max Min	Max Min	Min	Max Min	Min	Max Min	Min	Max Min	Min
WOODLICE	23	9	7	10	77	o,	8	6	g	7
EARTHACRAS	23	œ	25	12	24	13	88	6	23	10
SALAMMURSS	22	п	II	13	25	14	8	6	12	12
SINGS	24	11	22	12	26	13	83	10	24	14
REDMORMS	25	21	25	13	25	n	53	10	22	9

TABLE 8
PRECIPITATION (CM)

YEAR	MAY	JUNE	JULY	AUGUST	TOTAL
1972+	6.37	12.62	17.32	22.12	58.43
1973+	14.81	11.76	10.31	19.76	56.64
1975 ⁺	7.64	15.95	6.71	12.01	42.31
1976°	2.77	9.60	4.17	6.40	22.94
1977°	11.45	11.20	13.03	17.14	52.82

⁺ Taken at Glidden.

O Taken at WTF.

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